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DETACHED LEAF CULTURE

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Detached leaf culture is here defined as the maintaining of leaves in a living condition for various periods after detachment from the plant of which they were once a part. Culture is here used to indicate life or existence as used by Vickery *et al.* (279) and Mason and Phillis (170) and not to indicate indefinitely continued growth as used by Carrel (41) and White (295), since leaves have apparently never been cultured in the meaning of the word used by Carrel and White. In detached leaf culture, investigators may be primarily concerned with the development and function of the leaf itself, with some parasite on the leaf, or even with some hyperparasite on a parasite on a leaf. The use of detached shoots is arbitrarily included in this review, but embryo culture is excluded.

No claim to completeness of coverage is made, and the writer would appreciate having called to his attention any important publications in the field of detached leaf culture which are omitted from the literature cited for this review.

According to Mathuse (171), Madriola in 1652 reported the formation of roots and shoots by detached leaves. In 1754 Bonnet (26) published a book on the function of leaves, in which he reported in some detail many tests with detached leaves. While his interpretations of the functions of leaves are not acceptable to present investigators, his extensive data of the length of life of detached leaves held under various conditions of culture are still valid. Beginning about 1850 detached leaves came to be extensively used in studies of water absorption, transpiration, respiration and photosynthesis. The discovery by Boehm (24) in 1883 that detached leaves in the dark could form starch from sugar solution supplied to their petioles or to leaves floating on the solution was a

great impetus to the use of detached leaves in studies of organic nutrition. Farlow in 1885 (84) was one of the first if not the first to successfully use detached leaves for inoculation tests with parasitic fungi, and Mains in 1917 (159) was the first to demonstrate clearly by means of detached leaves the important relation of carbohydrate nutrition of the host to the development of a plant pathogen (corn rust). Detached leaf culture is now a convenient technique for many plant physiological, phytopathological and entomological studies.

PHYSIOLOGY OF DETACHED LEAVES AND EFFECT OF DETACHMENT ON NORMAL LIFE PROCESSES

Length of life and process of death. The early death of detached leaves is the greatest obstacle to their use. When leaves are detached from a plant they usually die within 24 hours if deprived of moisture, or within 24 days if adequate moisture is supplied, but some detached leaves have been kept for surprisingly long periods. Under the best environmental conditions used, the maximum length of life of various detached leaves has been reported as follows: potato—five months (138), mint—several months (127), mulberry—almost six months (26), ivy—six years (173), cherry laurel—50 days (17), *Evonymus japonicus*—18 months (171), corn—14 days (159), *Solidago rugoso*—four months (48), apple—nearly five months (91), tobacco—12 days (279), red clover—17 weeks (306), *Rudbeckia*—14 days (129), lettuce—two weeks (193), bean—130 days (78). The best of the scanty information on the comparative length of life of detached leaves of different species is given by Bonnet (26) and Clinton and McCormick (48).

The periods of viability are mostly given by investigators who apparently wish to emphasize the long life of detached leaves; the minimum and average periods, unfortunately, are usually not stated. It is noteworthy that all periods of long life are with dicotyledons, and it is known that detached leaves of monocotyledons are generally shorter lived.

While detached leaves usually die sooner than attached leaves they can sometimes be made to live much longer than normal leaves (127, 171, 172, 247). Waters (286) states that no leaf was tried which could not be kept five or six weeks by his method.

Detached leaves supplied with water decline in vigor over sev-

eral days and finally die, but, with few exceptions (11, 100, 279, 280, 324, 325), the process has not been described. Leaves with their petioles in water and their blades exposed to an unsaturated environment usually first show decline as a wilting of the blade, which is apparently associated with an interference with the translocation of water; this may be caused by plugging of vessels with micro-organisms or by host secretions. Leaves with their blades in a moist atmosphere and not attacked by micro-organisms usually show decline as a chlorosis starting with leaf margins, leaf tips or veins (100, 280) followed by a browning of the tissues (280), though the browning may occur without prior chlorosis. With cherry laurel leaves supplied with water in the dark, Audus (11) found the disappearance of chlorophyll, associated with an increasing rate of respiration, to begin in about 15 days and to be 75% complete in 35 days. Fungi began developing on the leaves in about 25 days. Hartt (100) found that detached sugar-cane leaves with their bases in sugar solution in the dark became chlorotic from the tip down starting with the third day, dried from the tip down starting with the sixth day, and by the 14th day were almost completely yellow with about half the leaf dried. Beginning of chlorosis may be associated with exhaustion of available carbohydrates (324) and with protein hydrolysis (177, 183, 325), and death (browning) may be associated with an accumulation of ammonia (280, 325). Frequently the death of the leaves is caused by their invasion by fungi and bacteria (48). Vickery *et al.* (279) report that destruction of chlorophyll was accompanied by a sudden loss of ether-soluble material and that completion of yellowing was associated with a reduction in protein hydrolysis. Exudation of water and filling of the intercellular spaces with water were associated with the death of barley leaves (324).

Growth and cell division. Leaves may increase in area and thickness after detachment (125, 146, 171, 220, 244, 247, 251, 290), and this growth is due principally to an increase in the size of epidermal, palisade and spongy parenchyma cells (146, 171, 247) with no increase in the number of these cells. The increase in area may be as much as five times in three to six weeks (244), and four-fold increases in length have been reported (125, 290). Schwarz (247) found greatest increase in area from rooted detached leaves. In cases where cell size was measured (146, 247), the length of the

cells was usually not so much as doubled after detachment, and it would therefore be of interest to follow the changes in cell size in leaves which show such great increases in size, as indicated above. Riehm (220) found that detached beet leaves grew at the same rate as attached leaves for the first three days, but after that the growth of the detached leaves declined rapidly below that of attached leaves. With nine species of plants he found that the average total increase in size of leaves after detachment ranged from a maximum of 64% with *Anthriscus* to a minimum of 2% with cyclamen. The maximum increase for a single specimen was 90% for onion. In 0.1 per cent Knop's solution or in 0.1 per cent sucrose, leaves grew more rapidly than in distilled water. As the concentration of the medium increased, the rate of growth decreased, but even on 4% Knop's solution or 21% sucrose the leaves remained healthy and some grew. Injury to leaves decreased growth. Most rapid growth occurred at 27° C., but the more rapid the growth the more quickly it ceased, so that the total growth was about the same at high or low temperature. Total growth as well as rate of growth was greater in darkness than in light. Growth at 150 mg. mercury was double that at atmospheric pressure. Growth was greater when the leaf was under water, and Riehm believed that any treatment that favored water absorption favored growth. Detached leaves which had ceased growing were induced to resume growth by cutting off the roots which had formed, by injecting the leaf with water or with sucrose solution, and by treatment with mercuric chloride or reduced atmospheric pressure.

Weintraub (290) cultivated detached oat leaves in a nutrient solution containing sugar and inorganic salts in darkness, and the detached leaves grew in length at about the same rate as attached leaves.

Schimper (244) found that nitrogen and calcium in the culture medium favored growth of *Pelargonium* leaves and that no growth of detached *Chenopodium* leaves occurred in the absence of nitrogen.

Detachment itself acts as a stimulus to growth for some leaves which have ceased to grow in the attached position (146, 171, 247). It would seem likely that this detachment stimulus might be associated with the normal pre-detachment turgor deficit in leaves

(278). While uninjured epidermal and parenchyma cells of the leaf blade do not usually divide after the leaf is detached, the division of cells in uninjured vascular tissue has been reported (63, 171, 247). According to Schwarz (247), cell multiplication of the vascular tissue was associated with the growth of the lamina and with the formation of roots.

Injury to leaves may stimulate cell growth or cell division, or both. La Rue (135) found that cell growth, generally without cell division, could be induced in detached *Populus* leaves in a moist chamber as a result of contact with the medium, or from contact with healthy leaves, with crushed intumescences, with extracts from *Rhizopus*, with pure indol acetic acid and with other treatments. La Rue suggests that leaf outgrowths showing cell enlargement require auxin for their formation.

At the cut surface of the petiole of detached leaves, callus may be formed by cell division prior to or without root formation (117, 133, 136, 156, 161, 207, 247, 251, 304). Roots may arise by cell division in the callus tissue or above it (117, 121, 156, 161, 251). Shoots may form at wounded or unwounded parts of detached leaves (96, 117, 158, 220). The presence of a dormant bud on a detached leaf (268) makes shoot formation more likely, but shoots may arise adventitiously on detached leaves (87, 117, 158, 287). The extensive detailed literature concerning the formation of roots and shoots by detached leaves and shoots is considered beyond the scope of this review.

Cell division may occur as a wound response in the lamina of leaves (12, 17, 133, 136), though the intumescences studied by La Rue on poplar (135) usually showed increase in cell size without cell division. La Rue (136) found that leaves of pteridophytes did not form callus on wounds, leaves of monocotyledons usually did not form callus on wounds, and dicotyledons frequently formed callus, though most species did not. He found that plants with great regenerative capacity usually formed callus.

Lamprecht (133) found that cell division in leaf wounds was greatest closest to the vascular tissue, especially the phloem, and that wound-stimulating substances could diffuse from the phloem and stimulate cell division of other leaves, including closely related species, occasionally other genera, but not of other families. Lamprecht believed that the capacity for cell division was reduced

as the size of the leaf pieces was reduced, and the smallest leaf pieces on which cell division occurred were 1.5 mm. square in the case of *Bryophyllum*. Priestley and Swingle (207) discuss the healing process at cut plant surfaces.

Isolated leaf cells may remain living up to four months and may increase in size (21, 52, 95), and Schmucker (245) reports division of an isolated mesophyll cell of *Bocconia* to have given about a dozen cells. This brief unconfirmed report of cell division is questioned by White (295) who reviews the numerous failures in the culture of individual cells and tissues of plants. The toxicity of tissue juices for cells of the tissue (204) is an interesting obstacle to the study of culture of cells of higher plants.

While growth and cell division of detached leaves are thoroughly established from the literature they have not been observed by most investigators. Vickery *et al.* (279) record that they observed no evidence of growth of tobacco leaves, and in the writer's tests where the leaves were usually held on 10% sucrose, no growth was noticed. The reasons for this apparent lack of growth in many cases is not clear, but are probably associated with species used and with cultural conditions.

Translocation. When leaves are detached from plants, translocation is reduced or almost eliminated, and this is responsible for important differences in the physiology of detached and attached leaves. In normal attached leaves there is a rather regular diurnal variation in water (62, 126), inorganic solutes (201), fresh weight (62), dry weight (51, 62, 226, 307), carbohydrates (51, 62, 169, 199, 226) and proteins (43), the highest values for water usually occurring at night and for the other materials during the day. Starch and other carbohydrates show the most striking and important diurnal variation. Sachs (226) showed that sunflower and beet leaves detached from the plant at 5 a.m. were starch-free, while similar leaves detached at 6 p.m. the previous evening and examined at 5 a.m. had an abundance of starch though less than similar leaves examined the previous evening. Sachs considered the slight decrease in starch in the detached leaves as due to respiration. With beet leaves he found that nocturnal translocation occurred within the detached leaf from the lamina to the veins, but when the lamina was freed from the veins there was only an insignificant nocturnal decrease in starch in the lamina.

Sachs discusses the relative value of morning and evening harvested foliage for fodder, tobacco, tea and silkworm food.

When detached leaves are floated on water or nutrient solution or placed with their petioles in solution, apparently only an insignificant amount of materials escapes from the cut portion of the leaf (50, 61, 226, 240). This elimination of translocation as a result of detachment is likely because of injury to and healing action of the phloem cells, which Curtis (50) has likened to the stopping of bleeding of animals due to coagulation of the blood at a wound.

Simon (251) considered that the growth of callus tissue at the cut end of leaf petioles was due to accumulation there of foods which could be translocated to that point but no farther.

Isolation of attached leaves by removing all but one of the leaves on a plant produces changes in attached leaves similar to the changes due to isolation by detachment, and these changes may be associated with translocation. Goos (92) studied the behavior of single leaves on plants after all other leaves on the same plants were removed or darkened. He found that the length of life, rate of growth, rate of assimilation and rate of translocation were greater for such single leaves than for control leaves on plants retaining their normal complement of functioning leaves.

Absorption of water. Detached leaves may absorb water through their injured or uninjured surfaces. Leaves in nature might be considered to be in a regular state of water deficit, for when they are immersed in water or placed with petiole in water and the remainder of the leaf in a moist chamber, they normally and promptly attain a greater weight than at the time of detachment (31, 45, 130, 157, 278, 279). Such leaves were generally collected during the day, and it is known that the water content of leaves during the day is less than at night (62, 126). The normally changing water content of leaves must be considered in determining water-absorbing power of leaves, for obviously less water is likely to be absorbed by a leaf which is already almost saturated with water. Bouissingault (29) observed that leaves collected in the early morning absorbed less water than leaves collected in the afternoon.

Absorption of water by leaves through cut petioles or stems is so frequently recorded and used in transpiration tests (172, 179) that the fact need not be further documented here. The progres-

sively slower rate of absorption and transpiration by some leaves with their petioles in water as compared with similar attached leaves is discussed under transpiration.

In tests of water absorption by the lamina, absorption through the petiole was usually prevented by holding it out of the solution (23, 26, 97) or sealing it with some material impermeable to water (31, 40, 129, 157), and water absorption or loss was determined by the weight change observed when normal or water-deficient leaves were weighed before and after exposure to moisture, with precautions to remove any adhering but unabsorbed water before each weighing.

Absorption of water through the uninjured surface of attached or detached leaves has been thus observed (23, 31, 40, 53, 54, 97, 104, 129, 157, 257, 293, 297, 329). Dandeno reviews much of the very early work on water absorption by leaves (53). It has not been clearly shown that this power of water absorption by leaves plays any important rôle in the water economy of normal plants, and Duchartre (68) and Ganong (89) failed to detect water absorption, but water absorption by uninjured surfaces is necessary or desirable in many detached leaf culture studies.

Absorption of water as water vapor has been reported (29, 40, 53, 157), but was not detected by Brierley (31). Burt (40) found that detached leaves or shoots lost water in a light moist chamber but gained water in a dark moist chamber, and this observation is closely associated with transpiration into a saturated atmosphere (55, 66). In cases where comparison was made of water absorption from free water and from water vapor, the rate was much slower for water vapor (29, 31).

Tests of water absorption by the lamina of leaves have been performed under a variety of conditions. Hales's classical demonstration of water absorption by leaves (97), since repeated by others, was to immerse in water one fork of a leafy forked branch, while the other fork was exposed to the atmosphere. The exposed leaves of this branch with its cut end out of water lived much longer than leaves of similar branches of which none of the leaves was in contact with water.

Henslow (104) detached leaves in the late afternoon, exposed them to drying conditions and allowed them to wilt for two hours, weighed them and exposed them under conditions of dew forma-

tion. At 7 a.m. the following day the free water was wiped off and the leaves were weighed. With 28 types or species, Henslow found that all regained their turgor, and the actual weight increases ranged from a minimum of 0.57% with *Berberis* to a maximum of 35% with grass.

Boehm (23) found that he was able to revive wilted bean leaves by immersing them in water when it was no longer possible to revive them by adding water to the roots of the supporting plant.

Ulchla (278) believed that water absorption by leaf tissues was injurious to the tissues, and that continuous growth of detached plant tissue was unlikely to be attained without further fundamental information on water relations. In harmony with this he observed that the injury to detached leaves decreased up to a certain point as the osmotic pressure of the substrate increased.

In contrast to the findings of Ulchla, Mason and Phillis (170) found that the longevity of cotton leaf discs was proportional to the amount of swelling which occurred when they were floated on a mineral nutrient solution. Leaf discs floated on a mineral nutrient solution increased their water content by as much as 170% in 13 days, while similar discs on pure water decreased in water content. They believed that salts in the nutrient solution increased the hydration capacities of the proteins.

Krause (129) found that the greater the water deficit in leaf tissues the greater the rate of water uptake. Also, thick, hard or waxy leaves absorbed water more slowly when detached than thin, delicate, non-waxy leaves. Generally the rate of water loss of detached leaves in the tested environments was much greater than the rate of water absorption in distilled water, but with *Phaseolus coccineus* he found that rate of water gain on immersion in water was about the same as the rate of water loss in wilting.

Prillieux (208) records a case of recovery from wilting of detached leaves in a moist chamber which was associated with a decrease, not an increase, of green weight.

Krause (129) observed that leaf hairs or cells at the base of leaf hairs showed more intensive staining with neutral red than other adjacent epidermal cells, and considered that these leaf hairs and adjacent cells might well be the point of entry of free water into the leaf when the leaf was floated on a solution. Marloth (165) also believed that leaf hairs could absorb moisture. Zam-

firescu (329) believed that free water entered leaves along the veins and readily penetrated to the vessels. These observations of Krause, Marloth and Zamfirescu are thus closely related, since in many species leaf hairs occur most abundantly along the veins.

Nakajima (187) found that water freed of air by heating or vacuum treatment was more readily absorbed by detached leaves than was water containing air.

Leaves immersed in or floating on water may become water-soaked, though no extensive treatment of this phase of detached leaf culture has been found. Sen and Blackman (249) observed that water soaking of immersed leaves occurred in darkness but not in light and believed that this was explained by the use of oxygen in the respiration of the leaf, while the carbon dioxide and nitrogen in the leaf were dissolved in water. Water soaking from a water spray is greater in light than in darkness, however, because of the opening of the stomata (64).

MacDougal (151) observed that dead leaf parts also absorbed water readily from a moist atmosphere, and this should be considered in studies of water absorption by leaves.

Transpiration. Detachment of turgid leaves may cause a sudden temporary rise in transpiration lasting only a few minutes (65, 86, 107, 118, 123, 132, 198, 250, 263) followed by a slow decline. This surprising increase in transpiration was first suspected from porometer values (57), later confirmed, and interpreted as due to a sudden increase in the opening of the stomata due to a release of water tension on detaching the leaves (56, 132). Such increased opening of stomata was not confirmed by direct observations (148) or by indirect determinations (129), though some investigators have shown a close correlation between transpiration and stomatal opening of detached leaves (148, 263). The sudden temporary rise in transpiration after detachment of leaves or shoots is usually not detected by ordinary weighing measurements when intervals of ten minutes or more between weighings are allowed, but is demonstrated by frequent accurate weighings, as with the torsion balance (107, 112, 123, 198). In many though not all cases accurately studied by Pfleiderer (198), no increase in transpiration after detachment was observed, but only a gradual decrease, as is commonly believed to be typical (38). A temporary rise in transpiration was observed, however, in each of 17 tests with three species described by Firbas (86).

If shoots were cut under water no temporary increase in porometer values (132) and presumably of transpiration occurred.

After the temporary rise in transpiration, if one occurs, detached leaves not supplied with an external source of water transpire at a progressively decreasing rate (86, 107, 123, 132, 198, 250, 263), which rate has been investigated mathematically (250) and correlated with leaf temperatures (323).

The amount of water which leaves may lose before wilting occurs apparently varies greatly with environmental conditions and with species. Bouissingault (29) observed no loss of turgidity when grape or sycamore leaves containing 66% water lost 13% of their water, but the leaves wilted if they lost 16% of their water. Knight (126), on the other hand, records extreme flaccidity of detached leaves resulting from a loss of 1% of their water content. Leaves may even recover from wilting while continuing to lose water (208). Even when detached leaves or shoots are placed with their stems in water after the stems have been cut in air or under water, a decline in transpiration usually occurs (29, 37, 88, 281) and probably continues until the leaf dies, unless the leaf forms callus and roots, in which case an increase in transpiration is indicated by Winkler (300). This decreasing rate of water loss for detached leaves makes them frequently unsuitable for transpiration tests, though there are great differences in the way different species respond.

Bouissingault (29) and Ganong (89) found that when detached shoots were subjected to increased hydrostatic pressure they wilted more slowly than shoots under normal pressure, but the fact that they wilted when attached shoots remained turgid indicates that some factor other than decreased root pressure was responsible for the decline of detached shoots.

Burgerstein (37) temporarily revived wilted detached shoots with camphor.

M'Nab (157) found that transpiration of detached shoots in different gases decreased in the following order: oxygen, air, carbon dioxide, nitrogen.

Biale (16) and Montermoso and Davis (182) showed that a diurnal rhythm of transpiration was exhibited by detached shoots or leaves in a constant environment.

Although guttation is generally considered as due to root pres-

sure (179), Vickery (280) records guttation on the lower surface of detached tobacco leaves presumably without roots.

Respiration. After detachment, leaves continue to respire at a rate similar to that of attached leaves, but with increasing time from detachment the respiration rate of detached leaves shows certain carefully described patterns, including a general decline in rate associated with exhaustion of carbohydrates (11, 28, 130, 194, 260, 324). Garreau (90) states that the respiration of detached leaves is similar to that of attached leaves, but in Garreau's data the comparable respiration data of detached and attached leaves are apparently not for the same plant species. In unpublished tests by the writer the rate of respiration of bean leaves, expressed as milligrams of carbon dioxide per hour per gram dry weight of leaves at the end of the test, was 1.19 ± 0.11 for detached healthy leaves, 1.52 ± 0.16 for attached healthy leaves, 1.76 ± 0.24 for detached leaves infected with *Erysiphe polygoni*, and 1.80 ± 0.23 for attached leaves infected with *E. polygoni*. These comparable values were secured from separate determinations within 15 hours of detachment of the respiration of nine single leaves of each type specified, and do not show clearly any difference between attached and detached leaves.

Decline in respiration after detachment is not constant, and temporary but consistent rises in rate in the course of the general decline in rate are usually observed (11, 130, 324). Audus (11) found the respiratory rate of cherry laurel leaves decreased from about 0.3 mg. per hour per gram of fresh weight at the beginning of the test to about 0.1 mg. at 240 hours after detachment, then rose to almost 0.3 mg. at 600 hours, then fell to 0.15 mg. at 740 hours, and rose again. The rise after 240 hours was associated with observed breakdown of chlorophyll, and the rise at 740 hours was associated with increased fungus growth on the chlorotic senescent leaves.

Yemm (324) reported that the respiration of detached barley leaves in the dark rose for approximately 12 hours after detachment, then fell until about 40 hours, rose or interrupted its rate of fall after 40 hours, and then fell rapidly from 140 to 240 hours as the leaves turned brown. None of the variations in carbon dioxide production could be correlated directly with concurrent changes in individual sugars, but in beans the carbon dioxide pro-

duced was accounted for by total carbohydrate loss for the first 30 hours. Later as carbohydrates became scarce the respiration rate decreased and the respiratory quotient decreased, presumably due to the utilization of protein in respiration. With barley less than 25% of the carbon dioxide produced from the 40th to the 140th hour could be accounted for by carbohydrate loss.

The greater the amount of carbohydrates available to detached leaves the greater their rate of respiration (130, 194, 289). Krotkov (130) found that the greater the initial sugar content of the leaves, as determined by cultural and seasonal conditions, the greater the initial rate of respiration. Palladin (194) found that at the end of the night period green leaves which have translocated their carbohydrates, respire as weakly as etiolated leaves. Weevers (289) with green leaves and Palladin (194) with green and chlorotic leaves found that respiration was greater for leaves on sugar solution than for leaves on water.

In the tests of Vickery *et al.* (279) the cellulose content of detached leaves remained unchanged up to 300 hours, and cellulose was therefore apparently not utilized in respiration.

Wounding (219) or mechanical flexure without wounding (11), either of which may easily occur in handling leaves for respiration tests, may increase the rate of respiration. Audus (11) found that bending detached cherry leaves in a standard manner increased their respiration by about 30% at the beginning of the test, but progressively less as the time from detachment increased.

Fungus infection generally and virus infection with less certainty, increases leaf respiration. This will be treated in more detail under culture of parasites. Iljin (115) considered that the increased respiration from fungus infection might be basically similar to the increase in respiration on wilting.

Laurie (139) believed there was a correlation between low respiration rate and long life in cut flowers. He found that hydrazone sulphate, phoroglucinol and resinol reduced respiration rate, and thought that proper addition of these to the water might prolong the life of flowers.

Only a relatively small part of the carbohydrates produced in leaves by photosynthesis is lost by respiration in a normal 24-hour day under ordinary growing conditions, and this appears equally true for attached or detached leaves. Ingen-Housz (116) was

one of the first to use detached leaves and come to this conclusion. Sachs in 1884 (226) indicated that the dry weight lost by respiration in one day was about one twenty-fourth of the dry weight gained by photosynthesis for detached leaves. Blackman and Matthaei (18) showed that over a range from about 10° to 35° C. the rate of carbon dioxide absorption per hour in light was about ten times the rate of carbon dioxide formation in darkness for detached sunflower and cherry laurel leaves. That factors other than rate of respiration in relation to available carbohydrates are important in determining the length of life of detached leaves in the dark, is indicated by the variation in the length of life of detached leaves of different species, and by the relatively short though significant increase in the length of life of leaves which follows the addition of supplemental carbohydrates to the substrate.

Photosynthesis. Most of our knowledge of photosynthesis, including the dry weight and gas exchange phases, has come from studies of detached leaves or shoots (18, 32, 33, 34, 83, 106, 109, 113, 114, 116, 212, 216, 226, 238, 239, 240, 254, 255, 256, 258, 260). The literature is too extensive, therefore, to be reviewed here, since photosynthesis is only one of numerous phases of detached leaf culture. We are intimately concerned, however, with the comparative photosynthesis of detached and attached leaves, and the photosynthesis of detached leaves over long periods, though unfortunately these phases have not been extensively studied, nor are the available data entirely satisfactory.

Sachs (226) found by his half leaf template method that detached sunflower leaves in full sunlight increased in dry weight at the rate of 1.64 g. per hour per square meter. By the same technique he found that attached leaves increased in dry weight at the rate of only 0.91 g./hr./m.² By adding an estimated translocation of 0.96 g./hr./m.² out of the leaf during photosynthesis of the attached leaves, Sachs's value for attached leaves became similar to that for detached leaves. The rate of translocation during photosynthesis was based on uncorroborated assumptions, however, and what was otherwise a careful comparison of attached and detached leaves cannot be considered adequate. Even the half leaf template method of Sachs has been severely criticized (258, 273).

Brown and Morris (33) confirmed Sachs's finding that the increase in dry weight of leaves in light was greater for detached

than for attached leaves, but they found that starch did not accumulate as rapidly in detached as in attached nasturtium leaves, a result which is difficult to reconcile with the above and with the idea that starch is formed when soluble carbohydrates become abundant.

Brown and Escombe (32) found that detached sunflower and *Catalpa* leaves assimilated about 82% more carbon dioxide per unit area than similar attached leaves, and suggested that the increased photosynthesis was due to the greater opening of the stomata on detached leaves. This explanation of the discrepancy has apparently not been established. In simultaneous determinations of assimilation by carbon dioxide absorption and dry weight increase for the same detached leaves, Brown and Escombe found that the rate, as indicated by dry weight increase, was about three times that indicated by carbon dioxide intake. Saposchnikoff (238), however, found less dry weight than expected on the basis of the amount of carbon dioxide absorbed. By careful methods, Smith (254, 255) has recently demonstrated concurrence of carbohydrate formation and carbon dioxide utilization by detached sunflower leaves.

Elimination of translocation from the leaf to other plant parts in the case of detached leaves is responsible for at least some of the observed differences in assimilation of attached and detached leaves. Saposchnikoff (240) and Ewart (83) have shown that the rate of photosynthesis of detached leaves decreases as the carbohydrates of the leaf are increased and that in strong light the carbohydrates of detached leaves may reach as high as 35% of their dry weight, which is more than that attained by attached leaves, and that photosynthesis entirely ceases in leaves with such high accumulations of carbohydrates. Such carbohydrate-gorged leaves assimilated actively again after being held in the dark for three days (83).

Protein synthesis and degradation. Because translocation out of leaves is practically eliminated by detachment, detached leaves are ideally suited for studies of the nitrogen changes of leaves, but there is little comparative information of the nitrogen changes of detached and attached leaves. Although it was early believed that light was necessary for protein synthesis in leaves (183, 244), others (183, 184, 267, 327, 328) have shown that detached leaves

supplied with sugar could synthesize proteins in the dark. Mothes (183, 184) found that young leaves synthesized protein from ammonium chloride and amides in the presence of adequate sugar, but as the leaves aged, as the sugar content decreased, or as the leaves were starved in the dark, protein synthesis ceased and protein decomposition began.

In detached leaves held in the dark and not supplied with supplemental carbohydrates, protein is gradually decomposed to amides and amines (45, 183, 260, 280, 325), to nitrates (154, 281) and to ammonia (183, 280, 281, 325), while the total nitrogen remains constant for long periods (61, 279). The liberation of ammonia is sometimes considered responsible for the death of senescent leaves (280, 325). Protein degradation is generally associated with carbohydrate exhaustion (61, 183, 266, 279, 281), but no sparing of protein by carbohydrates was detected by Yemm (325) or Chibnall and Sahai (45). The similarity of the nitrogen metabolism of detached leaves and of seedlings has been pointed out by Nightingale (191).

Takahashi (269) demonstrated that normal leaf protein but not tobacco mosaic protein was hydrolyzed to soluble nitrogen in detached leaves in the dark.

Chibnall (44) believed from his studies with detached leaves that asparagin was the chief form in which nitrogen in a form suitable for resynthesis of protein was translocated from one part of the plant to another, and Mothes (179) believed nitrogen was translocated in the form of amides.

Arisz and Oudman (8) found that absorption of asparagin by floating leaf discs of *Valisneria* occurred only in the presence of oxygen. The relative absorption was apparently greater from dilute than from concentrated solutions, for leaf pieces floated on 0.01% solution contained 0.36% asparagin after 72 hours, while leaf pieces floated on 0.66% asparagin contained 0.66% asparagin. Mothes (183) believed that the presence of asparagin increased the utilization of carbohydrates by detached leaves.

MECHANICS OF CULTURE

Different investigators have their favorite methods of culturing detached leaves, and these methods are in part determined by the peculiarities of the problem. All methods are extremely simple.

In studies of propagation, moist sand is commonly used as a substrate (127, 133, 265, 287). It has the advantages of being relatively free of decay-producing organisms, of holding sufficient moisture while allowing adequate aeration, and of excluding light from the root-forming cut end. The petioles are placed in the sand, and leaf blades are left above the sand surface. The bed of cuttings is usually covered so as to reduce but not to exclude light, and to maintain a high humidity. Root formation is sometimes inhibited by light (175, 247), but some leaf cuttings form roots readily in light (287). Techniques of propagation of leaf and other cuttings are given by Watson (287), and the general subject is beyond the scope of this review.

When regeneration is not desired, a common method for the culture of detached leaves has been in moist chambers, usually Petri dishes, on moist filter paper, with little consideration of light or carbohydrate supply (12, 19, 20, 227). Succulent leaves usually live only a few days under these conditions. Another simple method is to place the petioles of the leaves in water or nutrient solution, with the leaf blade exposed to ordinary air or the high humidity of a moist chamber (13, 14, 15, 18, 24, 42, 45). Leaves of Gramineae may be cultured with the base of the leaf immersed in the substrate in the bottom of a test tube and with most of the leaf exposed to the high humidity above the water (22, 100, 101, 277). Bonnet (26) laid the test leaves with most of the lower or upper leaf surface in contact with water but with the petiole and leaf margin projecting beyond the culture vessel and exposed to the atmosphere.

Floating of leaves directly on the surface of the aqueous substrate in a moist chamber is perhaps the most common method (8, 24, 48, 119, 286, 306). Hairy leaf surfaces are less likely to be wetted by the solution than non-hairy surfaces. If leaves are readily wetted by aqueous solutions the solution may creep over the leaf edge and on to the leaf surface which is facing up, and this seems to favor the growth of contaminants, to decrease the length of life of the leaves, and to reduce the growth of parasites, such as powdery mildews on the leaf surface. This tendency of the leaf surface to be undesirably wetted may be reduced by using a minimum amount of solution in the culture vessel, by using small culture vessels so that the culture solution is not unduly agitated in handling, by

laying the leaf lamina on the surface of waxed paper which is in turn floating on the culture solution while the leaf petiole is immersed in the solution, by laying the leaves on paraffined metal screens (180, 286) through which the petiole projects to the culture solution below, by laying the leaves on absorbent cotton soaked with the nutrient solution (71), by holding the lamina away from the culture solution by glass rods or rubber bands (48, 286), by inclining the culture vessel so that most of the solution is confined to a small area, and perhaps by other methods.

Entire leaves or leaflets are usually used, but for certain purposes leaf pieces are more desirable. For quantitative comparisons of photosynthesis, Sachs (226) and Brown and Escombe (32) used half leaves, and this half leaf method has been critically studied by Thoday (273). In studies of water absorption Phillis and Mason (200, 170) used leaf discs, and these would seem ideal for many quantitative comparisons using detached leaves, especially when twin leaves or leaflets or their equivalent are not available. Dundas (78) placed pieces of several leaves in the same Petri dish chamber. In this way he was sometimes able to put representative leaf pieces from each plant of an entire progeny of a cross in a single dish, while similar sets of leaf pieces could be placed in other dishes, and all could be inoculated simultaneously with different strains of a single pathogen (*e.g.*, bean powdery mildew or rust) or with different pathogens. There is of course a limit to which leaves may be subdivided and will still function suitably in detached leaf cultures. Hartt (101) found that cutting cane leaves into pieces one inch long did not decrease sucrose synthesis, but grinding inhibited the conversion of dextrose to fructose and the formation of sucrose. Detached leaf pieces have been used also in other studies (3, 4, 8, 21, 52, 95, 133, 155, 215, 301, 302).

Onion leaves usually die in a few days after detachment, but Ewart (83) found that detached onion seed stalks with their bases in water would live for weeks, a fact used in breeding onions (122). Harris, Drake and Tate (99) sealed the cut ends of detached onion leaves with paraffin, but were able to keep such leaves in a satisfactory condition for only three to five days.

For very small leaves or leaflets such as those of clover, Syracuse watch glasses are ideal culture vessels and can be stacked in large numbers so that each dish forms the cover of the dish below it.

For larger leaves Petri dishes are quite suitable, and for still larger leaves special vessels are necessary.

Leaves or leaflets are usually cut from the plant with scissors and transferred with forceps; slide forceps or those with curved tips are more suitable than straight forceps.

Mechanics of culture primarily concerned with avoiding contamination are treated under contamination.

Adequate comparisons of the utility of different methods of culture have not been reported, but little difference in starch synthesis has been found, whether the leaves floated on the solution or were supported with only their petioles in the solution (42). Schimper (244) reported that leaves with their petioles in water remained fresh longer than leaves in a moist chamber, but Mothes (183) considered floating leaves more satisfactory.

CONDITIONS AFFECTING LIFE OF DETACHED LEAVES

Moisture. Detached leaves die from desiccation a few hours after detachment in a normal environment, as indicated by data given under "transpiration." In detached leaf cultures the leaves are ordinarily in a closed chamber in contact with water or some solution which maintains an almost saturated atmosphere. They will live for long periods, however, if only part of one surface is in contact with the liquid while the petiole and most of the leaf surface are exposed to the atmosphere, as in Bonnet's tests (26). Clinton and McCormick (48) considered that high humidity of the air in the chamber lengthened the life of the leaves, but they and other investigators seem not to have adequately established this point. Hitchcock and Zimmerman (105) found that detached carnations with their stems in water lived two to three times as long in an approximately saturated atmosphere as at 80% relative humidity. On the other hand, Ulchla (278) believed that water absorption by the leaf was injurious to leaf tissues. Lamprecht (133) found that high humidity favored rooting of leaf pieces, low humidity prevented cell division, and that an intermediate humidity favored callus formation.

Temperature. The optimum temperature for maintaining detached leaves in good condition is lower than for the growth of entire plants, but is not well defined. Clinton and McCormick (48) had difficulty maintaining detached leaves long enough in

midsummer to secure infection with the rusts they were studying. Dundas (71) found that detached bean leaflets on 10% sucrose lived an average of 39 days at 8° C., 12 days at 25° C., and 7 days at 31° C. Hitchcock and Zimmerman (105) found that low temperatures increased the longevity of cut flowers of coriopsis, carnation and rose.

Boehm (24) found little difference in the amount of starch formed from sucrose at 10° to 20° C. In Chapman and Camp's studies (42), *Pelargonium* leaves showed increased starch formation from dextrose as the temperature was increased from 4° to 25° C.; the optimum was 25° to 38° C., the maximum about 50° C. Hartt (100) observed that absorption of dextrose or fructose by detached leaf blades of sugar cane remained about the same from 6° to 20° C., but increased from 20° to 40° C. The synthetic efficiency was 10% to 20% at 6° C., 72% to 83% at 20° C., 75% to 88% at 30° C., and 61% to 85% at 40° C. Leonard (143) found the highest synthetic efficiency at about 40° C., and sucrose was formed from dextrose at 50° C. Winkler (299) reported that the minimum temperature for conversion of sucrose to starch varied with the ecological type and season, and the maximum was about 45° C.

Roux (223) has described an unusual type of low temperature injury to detached leaves, manifested in loquat leaves as a browning and an increase in respiration.

The optimum temperature for the life of detached leaves may be a great deal less than that of any specific activity it is desired to measure, and it is likely that in many cases the higher the metabolic activity the shorter the life of the leaves. Considering both the life of the leaves and the leaf activity it is desired to measure, the optimum temperature for detached leaf cultures seems to be between 15° and 22° C. in most cases observed by the writer. It seems likely that the optimum temperature for detached leaves, like the maximum temperature for entire plants (137), might be lower in the dark than in light.

Light. Detached leaves exposed to light usually live longer than similar leaves in darkness, whether the leaves are supplied with water only (48, 78, 280) or with sugars (25, 71). Carbohydrates of leaves decreased more rapidly in the dark for leaves on water than for leaves on sugar solution (281). The two principal known

effects of light on detached leaf cultures are its effect in increasing carbohydrates through photosynthesis and its effect in reducing the growth of contaminating fungi and bacteria (167), though other effects are quite likely. Lepeschkin (144) has shown that light increases the permeability of leaves to inorganic salts and to dyes, though this was not corroborated by Zycha (332), and Phillis and Mason (200) have shown that light too low for photosynthesis increases the starch content of leaf discs floated on sugar solution. Light is not necessary for the formation of either carbohydrates or proteins in detached leaves, however, and many tests are advantageously conducted in entire darkness. Richm (220) found that detached as well as attached leaves grew more rapidly in darkness than in light.

Carbohydrate supply. If detached leaves are not cultured in light sufficient for adequate photosynthesis and if they are placed in contact with sugar solution rather than in contact with water, they usually accumulate more starch and sugar, probably show reduced exosmosis, remain living longer, have a higher rate of respiration, and maintain a more luxuriant growth of obligate parasites. This opportunity to manipulate the carbon nutrition of detached leaves independently of photosynthesis, first discovered by Boehm (24), was a notable contribution to plant physiology in itself and has been useful in many studies with detached leaf cultures.

Boehm (24) placed starch-free leaves in the dark with their petioles in sugar solution or with the leaves floating on the solution, and reported that by this treatment starch was formed in several species of plants, including four in which starch was not found in the leaves in nature. Leaves laid on 20% sugar solution formed more starch than leaves on 1% or 5% sugar solution, but there was little difference between 10% and 20% sugar. Leaves on 20% sucrose at 10° C. remained fresh for six weeks. Plants with roots but with cotyledons removed, lived longer in the dark or in a carbon dioxide-free atmosphere when the roots were immersed in a 0.25% to 5% sucrose solution than when the roots were immersed in water.

In most studies the path of absorption has not been considered, and cut leaves or leaf pieces have been floated on sugar solution or placed with their petioles in sugar solution. However, Schimper

(243) found that *Hydrocharis* leaves with their petioles held out of the sugar solution took up sugar solution through their laminae, and believed that the cells along the veins were especially active in absorption. Leonard (142), on the other hand, found no sugar absorption by beet leaves when only the laminae were in contact with the sugar solution and the petioles were in water. Waters (286) found that starch was deposited centrifugally from the bundles outward when cut pieces of leaves were floated on sucrose solution. The writer believes that sugar may be absorbed through uninjured leaf surfaces but that absorption occurs more rapidly through cut surfaces. Absorption of water and dyes by uninjured leaf hairs and by cells along the veins (129, 165, 329) would suggest a similar path of sugar absorption. The observation that clover leaflets increased in dry weight if the lower hairy surfaces were in contact with the sugar solution for 12 days, but decreased in dry weight if the upper relatively non-hairy surfaces were in contact with the solutions (307), would support the idea that leaf hairs facilitated sugar absorption in some way. Sugar may also be absorbed by roots and function to increase the growth of higher plants in light or in the dark (30, 128). If sugar is absorbed through the cut petiole, as in the tests of Vickery *et al.* (281), the sugar and water are absorbed in the same ratio as they exist in the nutrient solution, indicating that the sugar moves in with the transpiration stream, as would be expected.

Labile carbohydrates of leaves occur principally as starch, sucrose or dextrose, and absorbed or photosynthesized carbohydrates are usually converted to one or more of these. In dicotyledons, starch usually predominates, while in monocotyledons the reverse is true (24, 176, 197). According to Bokorny (25), starch is deposited when soluble carbohydrates reach a certain maximum which varies with species and other conditions. In the early work, formation of starch by destarched leaves was the criterion of sugar absorption, and it has been shown that green leaves may form starch from glycerine (1, 138, 176, 186, 237, 259), dextrose (1, 24, 42, 176, 186, 197, 259, 289), levulose (176, 197), mannitol (186, 197, 237), galactose (176), sucrose (1, 24, 176, 197, 200, 237, 243, 259, 286, 289, 299), lactose (176, 186), dextrin (186), inulin (1), melampyrit (186), maltose (176, 196), sorbitol (259), dulcitol (176) and perhaps humus extract (1), but not from erythrit

(176), oxalic acid (138), tartaric acid (138), tannin (138), acetic acid (138), xylose (192) or dioxyacetone (192).

Leonard (142) believed that starch could be formed in leaves from practically any sugar or sugar alcohol.

In what is perhaps the most comprehensive treatment of the subject, Nadson (186) studied starch formation from other carbohydrates in the detached destarched leaves of 19 dicotyledons, seven monocotyledons, four algae and one pteridophyte. He found that 24 out of 26 species tested formed starch from sucrose, 21 out of 22 from dextrose, seven out of 11 from lactose, 16 out of 26 from glycerine, six out of eight from dextrin, three out of 13 from mannit, and two out of three from melamprit. Only *Elodea* and onion formed no starch from any of the substances tested. Quercit, glycogen, gum arabic, calcium saccharate, tartaric acid, oxalic acid, malic acid, potassium tartrate and ammonia did not induce starch formation in any leaves tested. All materials which induced starch formation in Nadson's tests had alcohol radicals.

Next to starch, sucrose is perhaps the most important storage form of labile carbohydrates. Leonard (142, 143) found that sucrose accumulation in leaves was progressively less in leaves fed 6% sucrose, fructose, glucose, lactose, maltose and galactose, and no sucrose was formed from d'mannose, l'xylose, l'arabinose, d'sorbitol, d'mannitol or d'dulcitol. Hartt (100, 101) found that sugar-cane leaf blades absorbed large quantities of dextrose or fructose and that these were converted one into the other in the leaves, but that the concentration of each within the leaf remained low while the excess was converted to sucrose. Others, too, have studied sucrose synthesis from monosaccharides in detached leaves (150, 192, 282). Specificity of different sugars for leaves of different species are also discussed (102, 176).

The concentration of the test sugar is of course important in determining whether or not it is absorbed in detectable amounts by leaves. With sucrose solution, starch increase in the leaves proceeds parallel to sucrose concentration of the substrate (299) with a minimum concentration at about 0.1% (170, 299), an optimum concentration at about 10% but with little difference between 5% and 20% (24, 71, 78, 100, 176, 197, 286, 299, 306), and the maximum difficult to define but perhaps around 40% (78, 100, 197, 299, 306). Saposchnikoff (239) believed that the optimum

sugar concentration of the substrate was about the same as the optimum sugar concentration within the leaf, and Weevers (289) found that leaves could take up sugar from hypertonic solutions. With less favorable sources of carbohydrate a higher concentration is probably necessary to produce the same effect on leaf vigor or carbohydrate storage, and Meyer (176) observed starch formation by beet leaves on 20% glycerine, but lower concentrations were generally ineffective. Glycerine was approximately equal to sucrose in another study, however (277). Leaves which in nature form little or no starch require a higher sucrose concentration or more time for starch formation in detached leaf cultures than do leaves which normally form starch (243).

For leaves with their petioles in the nutrient solutions and their laminas exposed to the prevailing atmospheric conditions, the optimum sugar concentration of the substrate is lower than for leaves floating on the test solutions in moist chambers (24, 306).

The increased length of life of detached leaves resulting from the use of sugar solution instead of water as a substrate has been noted (22, 24, 78, 159, 277, 281, 286, 306). Sucrose and dextrose are more readily utilized by leaves than are other sugars (143, 176, 197, 277, 306), and sucrose is sometimes considered more favorable than dextrose (176, 197, 277, 286, 289, 306).

Detached green and chlorotic leaves utilize sugars about equally (24, 42, 100, 237, 289, 331), but albino leaves did not form starch from glycerine or mannitol (237) or from glycerine or sorbitol (259). Weevers (289) found that starch formed at lower sugar concentration in green than in chlorotic leaves. In wilting leaves starch is transformed to sucrose (2, 143, 246), and this transformation is independent of light (2) but can be prevented by killing the leaves at 72° C. before drying them (143) or by killing them with freezing, chloroform or toluene (261). A similar transformation occurs in drying potato tubers (284) and is therefore not peculiar to leaves.

Riehm (220) reported that sugar solution applied to leaves inhibited the formation of shoots but had less effect on root formation. Trelease and Trelease (277) found that chlorophyll was retained longer in leaves supplied with glycerine than in leaves supplied with the sugars tested by them. Winkler (299) and Phillis and Mason (200) noted that oxygen was necessary for the

absorption and interconversion of sugars by leaves and that the process was prevented by narcotics. Hartt (100) found that arsenite or selenite added to water in which sugar-cane leaves were standing in the light, prevented the accumulation of fructose but permitted accumulation of dextrose. Nurmia (192) compared the ascorbic acid contents of leaves held on water and on sugar solution substrates, and found no difference. Klebs (124) observed that degeneration of the chloroplasts occurred when *Zygnema* was cultured in sugar solution in the light, but such has apparently not been recorded for detached leaves of higher plants.

Pucher *et al.* (210) studied changes in organic acids in detached leaf cultures of rhubarb, and Vickery *et al.* (281) point out important correlations between organic acids and carbohydrate metabolism in detached leaves. Schimper (244) found that chlorophyll-free leaves were not able to make calcium oxalate. Fats of leaves are apparently less readily utilized than are sugars, for Chibnall and Sahai (45) found no significant changes in the fats of detached leaves of Brussels sprouts cultured for eight days with their petioles in water.

The importance and utility of carbohydrates in the nutrition of leaves is excellently illustrated by Spoehr's (259) total cultivation of albino corn by supplying sugar to the cut ends of the leaves of plants growing in a mineral nutrient solution.

The relation of sugar used as a substrate to the growth of parasitic fungi on leaves and to the development of contaminating fungi on leaves or in the culture medium will be treated in later sections of this paper.

Mineral supply. Detached leaves may absorb or release salts through their normal or injured surfaces, but these functions have not been shown to be important in detached leaf cultures. Absorption of mineral salts through uninjured surfaces of detached leaves was studied as early as 1754 by Bonnet (26), and has since been demonstrated by Boehm (23), Boussingault (29) and Dandeno (53), while Schimper (243) observed increased growth of detached leaves fed salts through their cut petioles. Mineral salts are fed to attached leaves in commercial practice in some cases by spraying the salts on the plants (298). In studies with detached leaves, however, most investigators have found no advantage in adding mineral nutrients to the substrate (78, 133, 240, 277, 280, 286,

306), some have found mineral nutrients injurious through their effect in increasing contamination (133, 306), and others have used mineral nutrients for detached leaf cultures without commenting on their utility (4, 91). In the absence of sugars in the substrate, Schimper (243) found that leaves lived longer in well water than in distilled water, and longer in complete nutrient solution than in well water. Riehm (220) noted that leaves grew more rapidly in dilute mineral nutrient solution than in distilled water, but in concentrated mineral nutrient solution, root development was inhibited, and in alkaline solutions roots were formed but not shoots.

Diffusion of mineral and organic salts from detached leaves into the substrate probably occurs continuously but is more rapid when the leaves are injured from whatever cause. Unfortunately for purposes of this review, most studies of this phenomenon have been with attached leaves. Arens (7) reports that when pure water such as dew is deposited on leaves, the cuticle becomes permeable to salts and other solutes and considerable exosmosis occurs. On many plant species dew drops were alkaline and exudation drops were acid. Distilled water at pH 5.6 became alkaline in a few hours when sprayed on foliage—more alkaline on upper than on lower leaf surfaces, and more alkaline on plants in sun than on plants in shade. The alkalinity was considered due to potassium, calcium and magnesium as well as organic substances, and Arens found that the mineral content of leaves could be reduced as much as 50% in 24 hours through exosmosis. Arens considered that the process of excretion functions as a regulatory mechanism whereby plants which have absorbed too great a quantity of minerals may decrease their salt content. Other evidence of loss of mineral salts through leaves has been presented (162, 177, 283), and Wallace reported a loss of up to 30% dry matter, 60% ash and 100% per cent potassium in four leachings in four days.

Brown's (35) study of the exomosis of nutrient substances from the host to the infection drop emphasizes the importance of such materials in plant pathology.

In the usual non-sterile methods of handling detached leaf cultures, the release of mineral salts from the leaves to the substrate is undesirable because of the effect of these salts in increasing the growth of saprophytic fungi and bacteria in the substrate and on

the leaves. This release of salts into the substrate is likely unavoidable, but can be reduced in quantity by avoiding injury to the leaves, by choosing vigorous leaves, and by using a substrate which will maintain the leaves in a vigorous condition. The growth of micro-organisms in the substrate made more nutritious by these mineral salts, can be further reduced by an approach to aseptic methods, and by occasional changing of the substrate solution. In aseptic leaf cultures, on the other hand, the above statements would not apply, and it is quite likely that the addition of nutrient salts to the substrate would be desirable (159, 290)

Other chemical supplements. In addition to sugars, which have been shown to have a decidedly beneficial effect on detached leaves when added to the substrate, and mineral nutrients, which are usually injurious under ordinary conditions of detached leaf cultures, there are a number of chemicals which would seem from indirect evidence to be of possible value if added to the substrate of detached leaf cultures, but which have not yet been adequately studied.

Jennison (120) reported that additions of boric acid, nitric acid, formaldehyde, potassium permanganate, charcoal and peppermint increased the life of some cut flowers. Laurie (139) found that many flowers kept longer if the receptacle contained copper metal. He also believed that hydrazene sulphate, sucrose, copper sulphate, sodium amitol and zinc powder might prolong the life of flowers. Sulfurous acid is said to prolong the life of cut flowers (6). Neff (188) studied the effect of some chemicals on the keeping quality of cut flowers, but his data are rather unsatisfactory. Hitchcock and Zimmerman (105) secured no marked improvement in the lasting qualities of several cut flowers by adding any of 50 chemicals to the water in which the stems were immersed. There was some evidence of improvement with potassium permanganate and ethyl alcohol, however.

Dawson (59, 60) found that glutamic acid, glutamic acid hydrochloride and nicotine hydrochloride added to the solution in which tobacco plants detached from their roots were standing, increased the absorption of solution, increased the life of the plants, delayed the loss of chlorophyll, delayed wilting and increased the nicotine content. The surface-active materials normally present on leaf surfaces (206) might themselves have an effect on the life of

detached leaves in liquid culture. In most of the above cases it is not clear whether the effect of the substances referred to is due to a direct effect on the leaves or flowers, or due to their effects on the growth of contaminating organisms.

Raber's report (213) of a possible relationship between hemoglobin and chlorophyll, as shown by the use of liver extract, would suggest the use of liver extract as a means of increasing the life of detached leaves, but in the writer's unpublished tests with non-sterile materials, contamination was excessive and the life of detached bean leaflets was not increased by adding liver extract to the water or sugar solution used as a substrate for detached leaves.

Species. Important differences in the time that leaves of different species may be kept in a vigorous condition in detached leaf cultures have been noted, but few generalizations can be drawn. Leaves of dicotyledons usually live longer than leaves of monocotyledons (48, 83, 286), and this might be because (a) monocotyledonous leaves usually store their labile carbohydrates as soluble sugars, while starch prevails in the leaves of most dicotyledons (24, 197), (b) detached leaves of monocotyledons form callus and roots less readily than do leaves of dicotyledons (96, 136) and (c) there is greater cut and injured leaf surface when most monocotyledonous leaves are detached than when dicotyledonous leaves are detached. In spite of this, Weintraub (290) has reported greater success in the culture of oat leaves than most other investigators have reported with dicotyledons.

The hardier leaves of shrubs and trees usually remain in a vigorous condition longer than leaves of herbaceous plants (26, 48).

Contamination and infection. The surfaces of leaves and fruits as well as many other outdoor surfaces are normally heavily infested with bacteria and fungi (39, 163, 253, 262, 270). Some of these micro-organisms grow well under the conditions normally maintained in detached leaf cultures and are usually associated with the decline in vigor and eventual death of the leaves (24, 48, 59, 78, 84, 133, 159, 183, 197, 220, 286, 299, 306). Though bacteria occur in numbers of 32,000 to 2,000,000,000 vegetative cells and 20 to 200 spores per gram of leaves (39, 253), they are apparently less injurious to leaves in detached leaf cultures than are fungi, though adequate proof of this is not available. Bacteria occur in large numbers in detached leaf cultures, but they usually do not

cause a visible clouding of the substrate until the leaf decays. Their occurrence and injurious effects in detached leaf cultures have been noted (279, 291), and the extensive development of bacteria in the vascular systems of detached leaves or shoots with their stems in water is believed to be partly responsible for the early wilting of these organs, but the bacteria occurring in detached leaf cultures have received little attention by most investigators. The apparent relative unimportance of bacteria, as compared to fungi, is possibly due to the acidity of the substrate (203) and its deficiency in nitrogen and other nutrients, as it has been observed that addition of nitrate increased bacterial growth in detached leaf cultures (306).

Adequate surveys of the number of fungus spores occurring on healthy leaves have not been found, though it is well known that any exposed leaf surface soon has a heavy population of fungus spores, and Marchal (163) lists about 80 species of fungi found on the surface of fruits. Which species are most important is not known, but *Penicillium*, *Rhizopus* and *Hormodendrum* are among the genera frequently found growing on the leaves when they start to decay. Under the abnormal conditions of detached leaf cultures, many fungi not normally considered pathogens will attack the leaves, and the distinction between true parasites and saprophytes is difficult (326).

The occurrence of fungus spores and organic matter on the leaves, the inherent vigor of the leaves, the nutritive value of the substrate, and light, are of importance in determining the development of fungus and bacterial contaminants in detached leaf cultures. Attempts to increase the length of life of detached leaves by reducing the initial population of micro-organisms have consisted of washing the leaves with water before use (48, 100, 133, 286) or with surface sterilizing substances such as mercuric chloride, alcohol, calcium hypochlorite, hydrogen peroxide and formaldehyde (78, 91, 183, 220), but only Mothes (183) indicates a definite improvement from such treatments. The reduction of established contaminants has been attempted by frequent changes of nutrient solutions (100, 183, 286, 299) and the trimming away of the cut or contaminated leaf edges (100, 183, 286). Other possibilities for reducing contamination which have been indicated but not properly explored are scalding or charring the cut ends before

use (120) and the addition of bacteriostatic and fungistatic chemicals (80) to the liquid substrate. Use of cleaned and sterilized glassware and other equipment (133, 286) are procedures which can be expected to eliminate nutrients favorable to the growth of contaminants and to kill micro-organisms which might otherwise be introduced into the cultures.

The longer life of young and medium aged than of old leaves, and the reduced contamination with greenhouse-grown as compared with field-grown leaves, as observed by Dundas (78) and the writer (unpublished), are probably related in part to the differences in the numbers of fungi, bacteria and organic matter on such leaves.

A high state of leaf vigor, though a rather indefinite quantity for which no definition will be attempted here, tends to reduce the growth of contaminating organisms on leaves and in the substrate. Leaf vigor may be influenced by conditions of plant growth before detachment, by time of day of detachment, by age of leaves before and after detachment, by carbohydrate and other nutrition of the leaves, by mechanical injuries to the leaves, by infection of the leaves with pathogens, and probably by many unknown factors, and it is of course difficult to separate direct from indirect effects. Dundas (78) found that leaves injured by *Diabrotica* beetles and by other causes, and leaves from plants grown on alkaline soil, were especially prone to contamination. Meyer (176) noticed that attacks by contaminating fungi were less when starch was formed in abundance in detached leaves. Yarwood (306) believed that, in general, the growth of the obligate parasites, *Uromyces* and *Erysiphe*, was better, and the growth of the facultative saprophytes, *Colletotrichum* and *Macrosporium*, was poorer, when clover leaflets were in a high state of vigor as influenced by the time of day the leaves were detached, the age of the leaves and the sugar content of the substrate, and he believes that these generalizations would likely apply to other leaves and micro-organisms. The greater the vigor of leaves the less their permeability (271), the less their permeability the less the diffusion of nutrients from the interior of the leaf to the substrate, the less the nutrient value of the substrate the less the growth of contaminating organisms therein, the less the growth of micro-organisms in the substrate the less the chemical injury to the leaves and the less the likelihood of these organisms attacking the leaves directly.

The nutrients added to the substrate of detached leaf cultures will affect directly the growth of micro-organisms present in the cultures or may affect the growth of organisms indirectly through the effect of the nutrients on the vigor of the leaves. Of the two carbohydrate nutrients commonly used, dextrose is more favorable than sucrose to the growth of most pathogenic bacteria (36, 178, 286) or to the organisms encountered in detached leaf cultures (78, 286, 306). Even when sucrose is used as a substrate it may be hydrolyzed to dextrose and fructose by autoclaving (149, 185), by the action of acids released into the cultures by the leaves (203), or by the action of micro-organisms in the cultures (36, 272). Waters' (286) observation that routine sterilized sucrose solutions were more favorable for the growth of contaminants in leaf cultures than were unsterilized sucrose solutions, might indicate some change other than hydrolysis, for Mudge (185) and McAlpine (149) found no hydrolysis of sucrose solutions till they had been autoclaved for more than 30 minutes. In the writer's tests, however, 10% solutions of C.P. or commercial sucrose, prepared with distilled water in routine cleaned glassware and sterilized by steaming for an hour or by autoclaving for five or more minutes at 15 pounds pressure, has shown much hydrolysis, as determined with Benedict's solution or with Fehling's solution. Similar unsterilized solutions have shown no hydrolysis or only a trace of it. Sterilization of sucrose or other nutrient solutions used as substrates for detached leaves is commonly practised, but usually without recorded desirable effect (133, 183, 197), and unsterilized sucrose has been used with satisfaction (78, 286, 306).

Addition of mineral nutrients alone or of mineral nutrients plus sugar to the aqueous substrate usually increases the growth of contaminating organisms in the cultures (78, 133, 306), though mineral nutrients have been used without recorded undesirable effect (91, 159, 286, 290). Yarwood (306) observed that the addition of magnesium nitrate to detached leaf cultures on sucrose solution favored the growth of bacteria in the substrate, while calcium phosphate favored the growth of fungus contaminants.

Light inhibits the growth of many moulds and bacteria (167), and several investigators have kept their detached leaf cultures in light (48, 286, 306). Usually only diffuse light is used, for if small closed dishes are exposed to full sunlight the enclosed leaves

are likely to be killed or injured by the heat (286), though no such injury occurs in open dishes.

Among the miscellaneous treatments for reducing contamination, Dawson (59) indicated that he delayed the decomposition of tobacco plants detached from their roots by chilling the culture solution to 5° to 10° C., and McGregor (153) believed that contamination could be reduced by the use of sun-sterilized water. Winkler (299) reports that in appropriate vessels containing phosphoric acid, leaves remained sterile for several days, and by frequently changing the solution, the leaf pieces on sugar solution were kept entirely free of fungi for several weeks.

The detached leaf may liberate at the cut surface, materials of a fatty nature which may be deposited as suberin in the walls of the intact cells (207). These fatty deposits inhibit penetration of the leaf by micro-organisms of decay, and the cork layer formed beneath the layer of fatty deposit is a further barrier to penetration by micro-organisms. The speed of formation of these fatty deposits may determine whether the wounded leaf decays or heals. If the cut surface is immersed in water decay is more likely than if the cut surface has access to air.

Few attempts have been made to maintain detached leaf cultures under aseptic conditions. Use of sterilized dishes and nutrients offers no special difficulty, but securing vigorous leaves free of fungi and bacteria is a major though not unsurmountable problem. By securing his leaf material from plants grown aseptically in test tubes, and by other precautions, Mains (159) was able to grow *Puccinia sorghi* on detached corn leaves apparently free of other organisms. Weintraub's (290) results in cultivating excised oat leaves in culture media containing sugar and inorganic salts are so outstanding that the writer feels that his cultures were also aseptic, though this is not specified in the abstract. The work of Bobiliooff-Preisser (21) was also presumably done under aseptic conditions.

For fundamental studies of detached leaves over long periods, aseptic cultures are necessary. For the few cases where cultures can not even be maintained long enough to secure the desired results with the methods used, aseptic cultures would likely offer a solution. However, for studies where detached leaf cultures are used only as a convenient tool for other studies, as is the case with most studies of detached leaves up to the present time, and

contamination does not interfere seriously, the extra labor involved in maintaining aseptic cultures would obviously not be justified.

Infection of detached leaves, like infection of attached leaves, usually reduces the length of life of the leaves. The distinction between infection and contamination made here is that infection is limited to those organisms normally pathogenic to the leaves, while contamination refers to the association of the leaves or substrate with organisms not normally pathogenic to the leaves. Dundas (71) found that detached bean leaflets infected with *Erysiphe polygoni* lived two to eight days less than non-infected leaflets. In the writer's studies several species of detached leaves artificially infected with different powdery mildews, different rusts, different downy mildews, *Phytophthora infestans*, *Colletotrichum trifolii*, *Macrosporium sarcinaeforme* and *Diplocarpon rosae* have consistently died sooner than comparable healthy leaves. No tendency of any of these organisms to grow into the nutrient substrate from the infected leaves was noticed.

Time of day of detachment and age of leaves. Leaves detached from plants toward the end of the light portion of the day have a greater content of carbohydrates (47, 51, 169, 179, 199, 226), of proteins (43, 183) and of minerals (201) than leaves detached just before the beginning of the light portion of the day. Since detachment eliminates or reduces translocation out of the leaf, it would be expected, therefore, that because of their greater amount of stored food, leaves detached in the afternoon would live longer than leaves detached in the early morning. This has been observed for apple (180) and for clover (306), though Dundas (71) noted little difference attributable to time of day of detachment for bean leaves on 10% sucrose. Hartt (101) reports that the ability of sugar-cane leaf blades to interconvert sugars varied with the time of day at which the leaves were removed from the plant, and this might indicate some diurnal variation in the enzyme system.

Young to intermediate-aged leaves usually live longer than old leaves, though there seems to be little quantitative information on this matter (78, 306).

Formation of callus and roots. The formation of callus (48) and of roots (173) is believed to increase the life of detached leaves. This is likely because of the wound healing (207) which normally precedes the formation of callus and roots.

UTILITY OF DETACHED LEAVES

Plant propagation. A large number of but probably not most species of higher plants may be reproduced by leaf cuttings. This subject has been reviewed (268) and will be discussed only briefly here. Extensive investigations of leaf cuttings have been made (96, 247, 265) and of the 1204 species tested by Hagemann, 389 formed neither roots nor shoots, 501 formed only roots, 25 formed only shoots and 289 formed both roots and shoots. Reproduction by means of leaf cuttings was more successful with dicotyledons than with monocotyledons or with gymnosperms. Hagemann considered the condition of the mother plant before leaf detachment to be important in deciding whether or not root and shoot formation would occur, and that light and nutrient conditions were relatively unimportant. Riehm (220) found that the more concentrated the nutrient solution the more limited the root development, and that alkaline solutions favored shoot formation but had no effect on root formation. According to Schwarz (247), infiltration of the leaf petiole with water or with diastase, or culture in the dark for six to eight days before transfer to light, favored root formation. Inclusion of an axillary bud or meristematic tissue on the leaf cutting makes shoot formation much more likely (87, 117). Detached leaves have the advantage over shoot cuttings that more individuals can be propagated from the same plant.

Physiology. Much of our knowledge of the physiology of leaves, which is a large phase of the physiology of higher plants, has come from studies with detached leaves. This is probably largely because of the great ease with which they can be manipulated and the results evaluated as compared with those of entire plants. Detached leaves have been successfully used in studies of water absorption (23, 26, 29, 31, 40, 53, 68, 89, 97, 104, 129, 170, 184, 278, 293, 329), of salt absorption (23, 29, 53), of transpiration (14, 16, 29, 37, 55, 56, 57, 66, 69, 86, 88, 103, 107, 112, 113, 118, 123, 131, 132, 157, 168, 181, 182, 198, 208, 225, 250, 263, 309, 319, 322, 323), of respiration (3, 4, 11, 18, 27, 28, 32, 33, 34, 61, 90, 93, 115, 116, 130, 141, 160, 164, 190, 194, 195, 205, 217, 219, 241, 242, 274, 276, 294, 303, 307, 319, 324), of photosynthesis (3, 18, 32, 33, 34, 83, 106, 113, 114, 116, 212, 216, 226, 254, 255, 256, 258, 260, 294), of carbohydrate synthesis in the absence of

photosynthesis (2, 24, 25, 42, 45, 100, 101, 102, 109, 124, 138, 142, 143, 176, 186, 192, 197, 200, 237, 238, 239, 240, 243, 246, 251, 261, 281, 282, 289, 299, 331), of protein metabolism (8, 154, 177, 183, 184, 244, 266, 267, 269, 279, 280, 281, 325, 327, 328), of wound responses (11, 12, 17, 63, 135, 136, 241), of translocation within the leaf (202, 226), of transplantation (133) and of electrical phenomena of leaves (236). They could probably be used in many other studies. Roach (222) used leaf injection of attached leaves in studies of mineral deficiencies of plants, and likely such studies could be performed to advantage with detached leaves.

In most studies adequate comparison of the results of attached and detached leaves has not been made to justify the belief (90, 104) that results with detached leaves apply in general to attached leaves, though the evidence available tends to support it. Detached leaves are uniquely superior to attached leaves in tests involving (*a*) weight changes, since it is impractical to weigh attached leaves; (*b*) gas exchange, since the petiole of attached leaves offers a means of gas exchange between the leaf and the stem, and since a closed system can therefore not be obtained with attached leaves; (*c*) chemical changes in which translocation is a complicating factor; and (*d*) comparison of leaves of the same plant, as of leaves of different ages. Detached leaves are superior to attached leaves in many other studies, especially if there is reasonable assurance that the results apply in general to attached leaves. The similarity in morphology and physiology of opposite leaves or leaflets offers an opportunity of securing more comparable test units with detached leaves than is possible with entire plants.

The two principal objections to the use of detached leaves in the study of the physiology of leaves are that they can frequently not be kept in a vigorous condition long enough for the desired tests in the desired environment, and that the results secured with detached leaves might not apply to normal attached leaves about which the information is desired. No attempt will be made to minimize the importance of these objections.

Culture of plant pathogens. Detached leaf cultures have been used as a substrate for the growing from spore to spore (total culture) of members of the Peronosporaceae (189, 313), the Erysiphaceae (5, 15, 70, 71, 72, 73, 74, 76, 78, 98, 119, 155, 209, 227, 228, 229, 230, 231, 232, 233, 234, 248, 277, 305, 306, 307, 308, 310, 311, 315),

the Uredinales (9, 10, 22, 48, 75, 77, 79, 84, 91, 159, 180, 264, 275, 285, 286, 306, 307, 312, 316), the Sphaeropsidales (306), the Melanconiales (20, 296, 306), the Moniliales (82, 174, 306) and also for the more limited culture of fungi where the extent of growth was less or was not clearly indicated (3, 4, 19, 35, 46, 49, 67, 85, 110, 164, 166, 190, 193, 203, 205, 214, 216, 217, 252, 291).

Purdy (211), Takahashi (269) and Woods (301, 302) have demonstrated multiplication or increase of tobacco mosaic in detached leaves inoculated after detachment. Purdy observed inclusion bodies but no macroscopic symptoms, while Woods secured clear macroscopic symptoms as well as inclusion bodies in leaves of *Nicotiana glutinosa*. In most of the above cases, the fungi have been primarily leaf parasites, but *Cicinnobolus cesatii*, parasite of powdery mildews, was cultured on powdery mildews which were in turn cultured on detached leaves (306).

Detached leaves have apparently not been used extensively for the culture of phytopathogenic bacteria, but Dr. P. A. Ark (unpublished) informs the writer that he has successfully used detached leaves for the culture of *Phytomonas pisi*, *P. phaseoli* and others. Detached leaves have undoubtedly been used, without specific mention in publication, in inoculations with many other organisms parasitic on plants.

In considering more specific types of plant disease problems, detached leaves or shoots have been used in studies of leaf penetration (19, 35, 49, 189, 235, 252, 316), comparative susceptibility of leaves to inoculation through upper and lower surfaces (20, 48, 286, 316), induced susceptibility (230, 233), effect of environment on susceptibility (15, 20, 48, 78, 85), effect of vitamins on susceptibility (209), effect of age of tissues on susceptibility (78, 306), effect of carbohydrate nutrition on susceptibility (78, 159, 203, 277, 285, 286, 306), host ranges and varietal susceptibility (48, 67, 70, 71, 72, 73, 74, 75, 76, 79, 98, 147, 180, 189, 227, 231, 248, 275, 310, 312), susceptibility of different leaf tissues (155, 230, 233), nature of resistance (155), inheritance of resistance (71, 72, 75, 76, 77, 310), comparative infective powers of different spore stages (48, 227), heterothallism of fungi (180, 308), formation of perfect or telial stages (22, 214, 285, 286, 308), occurrence of physiologic races of pathogens (71, 73, 74, 78, 79, 119, 227, 228, 232, 234, 275, 310, 312, 315), formation of toxins

by pathogenic fungi (291, 330), transpiration changes induced by infection (103, 309), respiratory changes induced by infection (3, 4, 141, 164, 190, 205, 217, 307, 319), photosynthesis changes induced by infection (3, 216), overwintering (231), diurnal cycles of pathogenic fungi (46, 309, 314), protective fungicides (110, 166, 313) and volatile fungicides (317).

Determination of the conditions governing the formation of teliospores by rusts is a good example of a problem in which detached leaf cultures played an important part in the solution. By manipulating the sugar nutrition of his cultures of several species of rusts on detached leaves, Waters (286) was able to demonstrate quite clearly that teliospore formation was usually stimulated by a decline in the amount of carbohydrates available to the leaves. That this principle was not universally applicable was apparent from Walters's observation that teliospore formation of *Puccinia triticina* was apparently favored by high carbohydrate nutrition of the leaves, an observation later confirmed by Bockstahler (22), also using detached leaves.

The most extensive practical use of detached leaf culture known to the writer has been made by Dundas (70, 71, 72, 73, 74, 75, 76, 77, 78, 79) who has maintained many physiologic races of bean powdery mildew (*Erysiphe polygoni*) and of bean rust (*Uromyces appendiculatus*) on detached leaves for several years and has tested the response of bean varieties, strains and segregating progenies to these many races of the two above pathogens in thousands of detached leaf culture tests. Such studies, with *Erysiphe polygoni* at least, would have been difficult or impossible by inoculations on entire plants.

Studies of the comparative development of pathogens on detached leaves and on entire plants indicate that in most cases the two methods give similar results in regard to susceptibility of hosts and virulence of pathogens, though adequate comparisons have apparently been made only with powdery mildews and with rusts. Clinton and McCormick (48) found that with aeciospores of *Cronartium ribicola*, 66% of 170 inoculations on detached leaves and 78% of 123 inoculations on potted plants were successful. With uredospores of *C. ribicola*, 57% of 169 inoculations on detached leaves and 57% of 54 inoculations on potted plants were successful. The ratings of disease intensity, while not clearly

defined, were usually less for detached than for attached leaves, however. Clinton and McCormick's tests on the detached leaves were made with water as a substrate, and it is to be expected that if sugar solution had been used, the rust development would have been greater on the detached leaves.

Fawcett (85) found that the maximum temperature for infection of citrus leaves with *Cladosporium* was higher for detached than for attached leaves.

Blodgett (20) concluded that inoculations of *Pseudopeziza* on detached *Ribes* leaves in moist dishes proved to be as reliable as those on potted plants.

Waters (285, 286) found that infection of rusts on detached leaves was sometimes greater than on potted plants, and that while it was difficult to maintain some rusts on potted plants during the short days of winter, it was relatively easy on detached leaves on sugar solution.

Dundas has shown a satisfactory correlation in the susceptibility of beans to powdery mildew (71) and rust (79) on detached leaflets and on field or greenhouse plants, but has observed that powdery mildew sometimes grew more luxuriantly on detached than on attached leaves (74).

Newton and Yarwood (189) reported that detached and attached leaves were equally susceptible to hop downy mildew.

Powdery mildews, like rusts, may develop poorly on greenhouse-grown plants during the winter months. To compare the growth of powdery mildew on detached and attached clover leaves, the writer (305) heavily inoculated leaves of six plants of red clover of varying susceptibility on January 10, allowed two leaflets of each leaf to remain on the plant and transferred one leaflet of each leaf to 10% sucrose in a Syracuse watch glass. The Syracuse watch glasses and potted plants were kept in the same greenhouse environment. After 11 days the powdery mildew showed luxuriant development on all detached leaves but relatively poor or no development on the attached leaflets. In sunny summer weather development on attached leaves was usually approximately equal to that on detached leaves. In tests of the host range of powdery mildew on *Trifolium* species, Yarwood (310) secured infection on detached leaves of some species on which no infection was detected on plants in the field or inoculated plants in the greenhouse.

Carbohydrate nutrition of leaves is apparently more important in the culture on detached leaves of the obligate parasites, the powdery mildews and rusts, than in the culture of other fungi pathogenic on such leaves. Salmon (227, 228, 229, 230, 231, 232, 233, 234, 235) and Clinton and McCormick (48) report good infection of powdery mildews and of rusts, respectively, without addition of carbohydrates to the substrate on which their detached leaves were cultured, but it was not until other work (159, 277, 286) with sugar solutions as substrata that the potentialities of detached leaves for the culture of these two important groups of obligate parasites became apparent. While the pioneer work was done by Mains, the most comprehensive treatment of the subject to date is by Trelease and Trelease. In their tests wheat powdery mildew (*Erysiphe graminis*) produced 100 or more times as many conidia when detached leaves, previously carbohydrate-starved and inoculated, were floated in the dark on arabinose, xylose, rhamnose, dextrose, levulose, galactose, mannose, sucrose, maltose, lactose, melizitose, starch, dextrin, inulin, glycerine or mannite, than when similar leaves were floated on water. The best mildew development occurred on leaves floated on dextrose, levulose, sucrose or melizitose. Sucrose solution is now the standard substrate for detached leaves used to culture parasitic fungi (70, 71, 72, 119, 174, 180, 203, 209, 275, 285, 286, 305, 306, 307, 308, 310, 312).

Except for the process of inoculation, the methods of culture of pathogens on detached leaves do not usually differ markedly from the methods for the culture of detached leaves where no pathogen is involved. Inoculation methods vary with investigators and according to the organism studied, but are usually basically the same as inoculation methods used on entire plants (70, 71, 159, 180, 189, 209, 227, 275, 305, 306, 307, 310). The advantages of the use of detached leaves over the use of entire plants for cultures of parasitic fungi have been pointed out (48, 306) but will be indicated here. Some are as follows:

(a) Economy of space. A series of inoculations involving several strains of different species of organisms on a series of different host plants may be incubated in a few cubic inches whereas similar tests with entire plants would require perhaps 100 times as much space.

(b) Economy of host material. Leaves of a single plant may

aphids have fallen from the leaves or crawled off the edge of the leaves and died in the substrate where they were overrun by bacteria and fungi. This objection, which is not serious enough to preclude the use of the method in all cases, is overcome by isolation of the lamina from the substrate, as described for thrips (13). Thrips are much more active than aphids and therefore more difficult to keep confined, and would not deposit eggs on parts of tobacco leaves (108), but can be maintained in adequate populations with or without special precautions to keep them confined to the lamina of the leaf. The cannibalism displayed by some insects makes culture in large numbers difficult, and this objection can be overcome in part by rearing individual insects separately.

It seems to the writer that there are many ways other than those mentioned where detached leaves could be used advantageously in the study of insects and related forms. Red spiders, for example, have laid eggs, hatched from eggs and increased by large numbers on detached clover leaflets on which clover powdery mildew was being studied. Detached leaves would offer opportunities for study of insecticides under conditions where the same insects could be kept under continuous microscopic observation on leaves without being mechanically disturbed, and would be especially useful in studies of volatile insecticides.

SUMMARY AND CONCLUSIONS

Unlike detached roots, detached leaves have not yet been induced to grow indefinitely, but individual leaves have been kept living for periods up to six years, in some cases longer than the normal life of attached leaves. Under the best conditions known, most detached leaves can be kept in good condition for about three weeks, which period is long enough for most physiologic studies. They carry on most of the functions of normal attached leaves including transpiration, respiration, photosynthesis and protein synthesis, are more easily subjected to detailed experimental manipulation than are entire plants, and much of our fundamental knowledge of leaf function has been derived from studies of detached leaves. Food materials of detached leaves do not escape in important amounts through the cut petioles, and detached leaves are therefore very suitable for studies in which it is desired to

eliminate translocation as a complicating factor. By culture of detached leaves in darkness, both photosynthesis and translocation are eliminated, and carbohydrate transformations can be advantageously studied. The floating of leaves on unsterilized solutions of commercial cane sugar is now the most common method of culture of detached leaves, but minor changes are desired for different species and types of tests. Detached leaves have served as a convenient substrate for the total culture of plant pathogenic micro-organisms, especially the obligately parasitic powdery mildews and rusts. Among the more important aspects of these two important groups of parasites which have been successfully studied by means of cultures on detached leaves are carbohydrate nutrition, effect of environment on disease development, host range, physiologic specialization, heterothallism, formation of overwintering stages, respiration and volatile fungicides. Use of detached leaves as a substrate for the study of insects is a relatively unexplored field which would seem to have advantages over conventional methods in certain studies.

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